when determining claim patentability. The Van Geuns ruling relates to a question of patentability under §103 of the statute, not §112. Therefore, the Examiner's reliance on the Van Geuns case is not applicable to the factual situation in the instant application. Nevertheless, the Examiner has suggested a cure for the aforesaid rejection of these claims, and other claims which recite the "pathologically abnormal" limitation. The Examiner has suggested the use of the phrases "cancer cells"; "hematopoietic progenitor cells"; and/or "epithelial cells", as the target cells being sought out. While disputing the Examiner's position, Applicants have amended the claims in the manner suggested by the Examiner so as to render moot this grounds for rejection.

Claims 1-14 have been rejected as being vague and indefinite due to the recitation of the phrase "well-defined zone", or "well defined annular zone" in these claims. Despite the fact that paraphernalia sold by Becton Dickinson and Company under the trademark "QBC" provides a "well-defined zone" in a container for analysis of blood cells, the Examiner disputes the fact that one skilled in the art would be able to determine what such a "well-defined zone" is. In the rejection the Examiner has pointed out that the specification in question refers to a well defined "free volume" which is formed between the container and the insert inside of the container. Applicants do not agree that the phrase "well defined zone", as that phrase is circumscribed by the modifying claim language is vague and indefinite, however, in an effort to moot this rejection, the claims have been amended to recite a "free volume", as seemingly suggested by the Examiner.

Claims 1-14 have been rejected as being vague and indefinite due to the inclusion of the terms "agent" and "material" in these claims. As noted above, Applicants have amended the claims to restrict the target cells being sought out by the method, and therefore Applicants do not believe that the terms "agent" and/or "material" are vague and indefinite relative to the now-recited target cells. The specification is quite clear as to what agents or materials could be used to accomplish the desired results of the methods as presently claimed.

Applicants note that the rejection based on the "stains" and/or "colorants" claim

B

terminology has been withdrawn.

The rejections based on the inclusion of the phrases "clarified morphology" and "abnormal morphology" have been addressed by restricting the categories of target cells being assayed. The presently worded claims simply require that nucleated cell morphology be clarified and examined. The claims thus merely require that the colorant be able to morphologically distinguish cells on the basis of cellular morphology from other non-cellular material that might be colored by the colorant.

The rejections based on the use of the term "differentiate" have been withdrawn.

The rejection based on a lack of clear correlation step was maintained for Claims 3 and 6. Claim 3 has been amended to relate to a method for identifying circulating cancerous epithelial cells in the preamble, and the last step recites identifying epithelial cells having cancerous morphology. The present wording of the claim is believed to render the "correlation" rejection moot. Claim 6 has been amended to be restricted to a p/a test for cancer cells in blood, and the last step has been amended to recite the step of determining whether there are any cancer cells present in the sample. The present wording of this claim is believed to render the "correlation" rejection moot.

The rejection of Claims 2 and 14 based on a failure to describe when the blood sample is centrifuged has been withdrawn.

The rejection of Claim 2 based on the allegation that the term "percentage" is vague and indefinite. Claim 2 has been amended to eliminate the allegedly vague and indefinite term.

The rejection of Claim 3 based on the order of the steps recited has been withdrawn.

The rejection of Claims 4-6 has been maintained despite the amendment to recite "formed constituent components" of blood. The Examiner has suggested the addition of "cellular" after "formed". Blood has cellular constituents which are formed, but it also

contains platelets which are formed constituents, but are not cellular. Claims 4-6 have thus been amended to recite formed cellular and platelet constituents or components. This amendment is believed to render the rejection of these claims moot.

The rejection of Claims 4-6 based on the allegation that the phrase "insert that is operable" is vague and indefinite has been maintained. Part of the Examiner's argument goes to the prior recitation of a "well-defined zone" in these claims. This phrase has been deleted and the phrase "free volume" is presently recited in the claims, as suggested by the Examiner. A second part of the argument proffered by the Examiner relates to the use of a trademark, i.e., QBC, in a patent application. The Examiner has cited Ex parte Simpson (Bd. App. 1982) as authority for her argument. The cited case stands for the proposition that when a trademark is used in a claim, its use renders the claim indefinite. In the instant case, the trademark QBC does not appear in any of the claims, thus, reliance upon Simpson is not well taken. The Examiner's attention is directed to the fact that the mark QBC is not the only way provided in the application to determine the nature of the insert. There are also patent numbers which are cited that describe a system which has an insert that can function in the manner claimed in these claims.

The rejection of Claims 4-6,9 and 13 based on inconsistencies between the claims and their claimed objectives has been withdrawn.

The rejection of Claims 7, 9 and 13 based on the use of "axially elongated insert" has been withdrawn.

Regarding the rejection of Claim 10, the term "microscopical" has been deleted, and the term "microscope-like" has been inserted. Applicants do not agree that the metes and bounds of this term, or its predecessor term, cannot be determined by one skilled in the art. The components of such an instrument are clearly shown in the drawings and clearly described in the specification. The instrument in question is most certainly not "extremely tiny".

The rejection of Claim 14 based on the use of the phrase "signal result" has been

maintained. The Examiner has suggested verbiage which would obviate the rejection, however the suggested verbiage is deemed to be too limiting since it does not encompass a negative signal result.

The broad rejection of Claims 1-14 as being non-enabled due to the fact that the metes and bounds of the claims cannot be determined has been clarified in the final rejection. The undersigned now interprets this rejection as being based on an allegation that the full scope of the claims in question is not enabled by the specification as filed, therefore, the specification is deemed non-enabling for certain of the claims. The Examiner has clarified her position by pointing out that the specification as filed does not enable one to detect any and all pathologically abnormal nucleated cells which may be present in a blood sample, but merely enables the detection of epithelial cells, and hematopoietic progenitor cells. It is believed that the claims have now been sufficiently amended to render this grounds for rejection moot.

It is submitted that most, if not all of the §112 rejections put forth in the final rejection in the parent application have been rendered moot by the amendments presented herein.

THE §103 REJECTIONS

In the final rejection, Claims 1-14 categorically rejected as being obvious over the combination of Levine '217 and Rickman in view of Nagy et al and Goldblatt et al.

In analyzing the Levine et al reference, the Examiner notes that differentiation of target cells by epitopic-specific labeling is described, that gravimetric separation of target cells from non-target cells is described, and that quantitation of target cells is described. What the subject reference actually teaches is the differentiation of target cell bands by epitopic labeling; the use of specific gravity-altering components such as liposomes or plastic beads that also bind to the target cells and which cause the target cell bands to gravitate into the red cell layer of a centrifuged sample of blood, or into the plasma layer of the centrifuged sample of blood; and the quantitation of target cell bands. Thus, Levine et al requires the use of components that will attach to target

B

blood cells, and alter their natural specific gravity so that the target cells, when centrifuged, will gravitate to a location in the blood sample which is off set from the location of other non-target cells that have the same specific gravity. If one were to follow the teachings of the Levine et al patent, none of the target cells would gravitate to the area in the centrifuged blood sample which is occupied by the buffy coat constituents. The Examiner has conceded that Levine et al does not detect individual cells. Levine et al does detect target cell bands.

The Examiner characterizes Rickman et al as teaching that processing blood samples with the QBC tube is quicker and as sensitive as processing blood smears in the differentiation of target cells from other blood cells. This assessment of the Rickman et al teachings is clearly erroneous. Rickman et al does not suggest that the QBC tube can be used to differentiate target cells from other blood cells. What Rickman et al does tout is the use of the QBC tube to detect malarial parasitic infestation of a blood sample. The Examiner then notes that Rickman et al saw individual cells, apparently other than malarial parasites, in the compressed layer of the blood sample, citing Col. 1, page 69, the last line of the second full paragraph. This is the only reference to individual cells, other than malarial parasites, in the entire Rickman et al article. We submit that the Examiner is taking this single reference to cells out of context and suggesting that Rickman et al is detecting blood or other human cells in the blood sample being examined. This is clearly not true. Rickman mentions in passing that individual leukocytes can be seen, but does not see any particular utility in this observation.

Rickman et al describes a procedure for detecting the presence of malarial parasites in a centrifuged sample of blood. All that the Rickman et al publication suggests is that the use of the QBC paraphernalia to detect malarial infestation of a blood sample is quicker than the use of blood smears. Applicants do not contest this fact. There are hundreds of published articles extolling the virtues of using the QBC technology to detect malarial infestation of blood. Rickman et al does not suggest the desirability of detecting any other component of the blood sample being examined. Enclosed is a brochure which describes and illustrates the ability of the QBC system to detect and differentiate different types of malarial infestation in a blood sample. Pages 3 and 4 of

this brochure have photographs of an uninfested blood sample and an infested sample. The malaria appears as small green dots in the red cell layer. The photograph on page 4 is what Rickman et al is describing. It illustrates just how well one can see the individual leukocytes in the QBC tube, and also illustrates the bands of different types of leukocytes in the tube. These cell bands are what is being quantitated in the standard QBC test, not the individual cells. Note that the malarial parasites are always in the red blood cell layer. This brochure is submitted merely for the purpose of making it crystal clear to the Examiner exactly what Rickman et al is describing and seeing.

Nothing in either the Levine et al or the Rickman et al references suggests the desirability of microscopically examining individual cells within a space around the float which is used in the QBC procedure.

The Examiner concedes that neither Levine et al nor Rickman et al suggests the examination of a centrifuged blood sample for the presence or absence of cancer cells. Likewise, neither of the references suggests the desirability of differentially labeling hematopoietic progenitor cells or epithelial cells so that they can be distinguished from other nucleated cells in a centrifuged blood sample.

Regarding the detection of cancer cells in circulating blood, the Examiner cites Nagy and Goldblatt et al. The Examiner characterizes Nagy et al as providing a teaching of the detection of cancer cells from smears of nucleated blood cells that have been centrifuged and stained with a morphometric stain. A careful reading of the Nagy article indicates that the examination of smears of nucleated cells taken from a centrifuged sample of blood derived from subjects known to have cancer yielded a very low percentage of confirmed cancer cells. In a total of 79 known cancer patients whose blood was analyzed by scanning smears of the nucleated portion of a centrifuged blood sample, only 3 scans indicated the presence of cancer cells. This indicates only a 3.8% positive confirmation of cancer cells in the circulating blood of subjects known to have cancer. (See the Summary column of the Nagy article). This finding would indicate that the sampling of the nucleated blood cell portion of a centrifuged sample of blood is not a reliable procedure for the detection of circulating

cancer cells. Note further, that Nagy suggests only the use of AO as a stain for detecting cancer cells in blood. With all due respect, Applicants believe that Nagy, taken as a whole, suggests that the analysis of the nucleated portion of a centrifuged sample of whole blood that has been highlighted by a stain is not a reliable procedure for detecting cancer cells in blood.

Goldblatt et al has been cited as containing a description of a plethora of methods known to detect cancer cells in a sample of circulating blood of individuals known to have cancer tumors. Goldblatt et al, first column, page 6, state that they had reviewed studies concerning more than 3,000 patients and 2,000 controls in order to determine whether there is any biological significance of circulating cancer cells. Goldblatt et al go on to state that: "No positive significant correlation could be found between cell recovery and the various parameters of experimental design and methodology used, such as patient selection, technique of cell concentration, stain, single sample volume and preparation, be it smear or filter.". All of the techniques for detecting cancer cells in circulating blood, be they flotation, sedimentation, lysis, or what have you use either smears or filtration to try to detect the cancer cells. None of them described a technique wherein the cancer cells can be detected *in situ* in a centrifuged sample of blood. The summary section of Goldblatt et al quotes the Circulating Cancer Cell Cooperative as stating that: "At present there is insufficient basis for dependent clinical application of these observations (of circulating cancer cells) to diagnosis, prognosis, or choice of therapy.". In the final rejection, the Examiner opines that: "That cancer cells in the circulation have diagnostic value has been well established since the publication date of Goldblatt et al, as evidenced by Nagy et al.". It is respectfully submitted that the two articles relied on by the Examiner in making this statement indicate, quite to the contrary, that the presence of cancer cells in circulating blood, is a phenomenon which requires further analysis and investigation in order to establish some diagnostic value. Note that the Goldblatt et al review comes to a completely opposite conclusion than that espoused by the Examiner.

The Examiner states on page 17 of the final rejection of the parent application, that: "+++ the detection of cancer cells was +++ known, and that said cells were separable by density centrifugation, i.e., one would know where to find them in a centrifuged

B

sample.". Nevertheless, it is quite apparent that none of the prior art cited by the Examiner has ever suggested that one should, or even could locate and identify individual cancer cells, or epithelial cells, or hematopoietic progenitor cells in a centrifuged sample of blood.

In summing up the final rejection, the Examiner alleges that the four cited and applied references, taken as a whole would give one of ordinary skill in the art "a reasonable expectation of success" in his or her efforts to identify circulating cancer cells in a sample of centrifuged blood. We submit that the combination of references cited would not instill in one of skill in the art an expectation of success in this endeavor for the following reasons.

We enclose an article published in April, 1998 entitled: "Detection and characterization of carcinoma cells in the blood" by E. Racila et al. This article describes a process for detecting circulating cancer cells in a sample of blood, which process involves magnetic separation of cancer cells from the other components of the blood, and then flow cytometric analysis of the magnetically separated material in order to determine whether the separated material contains cancerous epithelial cells. Table 1 on page 4591 of the article indicates that a 10 ml sample of blood taken from persons having different types of cancerous tumors was shown to contain between 1 and 140 epithelial cells. The Examiner has cited Rickman et al for the proposition that individual leukocyte cells can be observed in the QBC system. A 10 ml. sample of normal human blood will contain an average of about 50,000,000 leukocytes. In a subject who has been infected by malaria, an average infection will involve about 3.0% of the red blood cells. In a 10 ml. sample of infected blood, there will be a total of about 50,000,000,000 red blood cells, and about 1,500,000,000 of the red blood cells will be infected with malarial parasites.

It will be noted that there are staggering differences between the number of leukocytes in a 10 ml. sample of blood, the number of malarial parasites in a 10 ml. sample of blood as compared to the number of cancer cells in a 10 ml. sample of blood. It is submitted that the differences in the various discussed cell populations in a 10 ml. blood sample are so dramatic that the fact that one could detect individual cells

B

(leukocytes), *in situ*, in a centrifuged blood sample that would be expected to be present in a population of 50,000,000 in the 10 ml. blood sample; or that one could detect cells (infected red blood cells), *in situ*, in a centrifuged blood sample that would be expected to be present in a population of 1,500,000,000 in the 10 ml. blood sample, does not give rise to a reasonable expectation of success in using a similar technique to try to detect cells, *in situ*, in a centrifuged blood sample which would be expected to be present in a population of at most 140 and as little as 1 in a 10 ml. sample of blood.

With respect to the detection of circulating cancer cells in a blood sample, the prior art indicates that the cancer cells must be separated from the remainder of the blood sample either by filtering, or by examination of concentrated cell smears taken from the blood sample, or by magnetic separation followed by flow cytometry analysis. Nothing in the prior art suggests that circulating cancer cells, which are extremely rare events, could be detected *in situ*, in a centrifuged sample of blood.

It is respectfully submitted that the cited prior art taken in concert does not render obvious the subject matter of the claims in this application. Applicants would seriously consider further limiting the claims to the detection of circulating epithelial cells and/or hematopoietic progenitor cells in order to accelerate allowance of this application.

Respectfully submitted,

William W. Jones
Attorney for Applicants
Reg. No. 24,607
6 Juniper Lane, Madison, CT 06443
(203) 245-2418

4-6-00

B